



Morphological studies on Digenea in Sidhi district

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Abstract

The species identification in digenetic trematodes is based mainly on the morphology. It is relied upon a series of measurements based upon maximum and minimum records on the length and breadth of the parasite and the dimension of various organs patterns. A successful parasite, during its evolutionary conditions, has already developed flexibility and therefore, generally takes the first course and adapts accordingly. These modifications lead to the changes in the morphological characters of the species.

Keywords: morphological, digenea, Sidhi district

1. Introduction

Life is the most fascinating phenomenon encountered by the human brain. It is close to the human psyche, since we are all living beings. We see it all around as plant and animal life with an enormous diversity. In its capacity to organize and reproduce it is completely different from all other inanimate phenomena. It was thus necessary to invent the concept of a mysterious "life force" to distinguish living organisms from all other. The biological wealth of the country can thus be converted into economic wealth leading towards a sustainable Biofuture-the great challenge as we move into the next millennium.

The survey of literature on the helminthology reveals that since the remote past the trematodes are an important groups of parasites and consequently their study is of great significance both from academic and economic points of view. Experimental investigation in trematodes is particularly a challenging task, for the establishment of specific in the laboratory depends on the supply of the parasites from the hosts. This group of our biological science is now going to be a very difficult and pains taking job. In this branch of helminthology, there are so many difficulties in addition on the non-availability of the hosts; sometimes we do not find the infection or many a times, the trematodes are not available for restudy.

Limit of intra specific variations in digenetic trematodes is a burning topic for the present helminthologists. No sound conclusions have yet been derived to understand the limit of intra specific variations. Many of the taxonomists have made mistakes of giving too much importance to small differences and have created species on the basis of such minor characters. It is increasingly felt to review again the whole group systematically and to give suggestions for defining the limits of the specific variations of a particular species. The author, most humbly has selected three species of birds for the group Digenia which are easily available from the region. The available species under the study are rigorously studied for their morphology. The guidelines and suggestions are also incorporated in the chapter of the general remarks of this

paper.

Limited published information is available on the digenetic trematodes from Vindhya region and adjoining areas. During the course of present studies of helminth parasites, plenty of data had been piling up from various areas. The present work deals with the study of digenetic trematodes harbouring mostly the groups of vertebrates (fishes, amphibians, reptiles, birds and mammals) and their ecology of Sidhi Distt. Helminthological researches in India have been conducted by several investigators and contributions have been published on Indian digenetic trematodes.

In spite of the attention paid by several helminthologists and so much work done, our knowledge of digenetic trematodes has still a large gap to be filled because of numerous species and genera running into hundreds and diversity of hosts which are involved at one stage or other in the life histories of the parasites. Our knowledge can only become complete gradually, as more and more work is done in this field.

2. Material and Methods

Sidhi of Rewa Commissionary centre situated at 24⁰32'N latitude and 81⁰18'E longitude. It is 365.7 meters above the mean sea level. It is situated in the South-East corner of Madhya Pradesh. North border touching the Uttar Pradesh, east having U.P. and West by Shahdol District. All these four districts together form Rewa division of Madhya Pradesh. The experimental site is quite rich in its fauna and flora with beautiful climate.

For the Morphology the flattened parasites were fixed in aqueous or alcoholic Bouin's fluid, 90% alcohol or 5% - 10% of formaline. The alcoholic Bouin's gave the better result. The trematodes are fixed in Bouin's for about 18 hours duration, washed thoroughly to remove the fixatives bleached in chlorinated water or chlorinated alcohol.

For preparation of whole mounts, the precautions were taken to avoid over or under pressure. For bigger parasites the flattening was made between two slides with blotting paper but in the case of small and delicate flukes the slide and cover slip only serve the purpose. The fixatives are used as per

requirement.

Bouin's fluid is the best fixative for all general purposes. Formaline 10% may be used when Bouin's fluid is not available. The specimens after fixation should be washed in water for a few hours and then transferred to 50% alcohol. They should be kept in 50% and 70% alcohol respectively over night. 70% alcohol should be changed once after 24 hours to remove the yellow picric acid colour of Bouin's fluid. Different stains were used from time to time for staining the trematodes, viz. Ehrlich's Haematoxylin, Gower's carmine, Paracarmine, Acetocarmine, Mayer's carmine and sometimes Borax carmine Formulae given. The worms were dehydrated through increasing grades of alcohol (30%, 50%, 70%, 90% and 100%), cleared in xylol, clove oil, cedar wood oil, Benzene, mounted in Canada balsam avoiding the air bubbles, dried at about 40-45°C, labeled then studied under Meopta binocular research microscope. Microphotographs were taken with the help of Meopta attachment camera. The films were developed in the formula G. 214 and Kodak formula for 3-5 minutes time. The drawings were made by camera lucida. In morphological study all measurements were taken in millimeters.

3. Observation

Family: Echinostomatidae Poche, 1925: Head collar surrounding oral sucker dorsally and laterally present. Collar spines in single or double dorsally continuous or interrupted row with or without corner groups. Head collar without spines in pseudochinostomum odhner. Acetabulum large, usually preequatorial. Sometimes equatorial or postequatorial. Oral sucker small, occasionally rudimentary or absent pegasomum Ratz. Oesophagus with cells i.e. pseudoesophagus: Caeca reaching near posterior extremity, sometimes opening into excretory vesicle (Chaunocephalus Deitz). Genital pore preacetabula. Testes usually tandem sometime diagonal or juxtaposed. Cirrus sac spaceacetabular anteriodorsal to acetabulum or extending behind the latter exceptionally absent (Pelmastomum Dietz). Ovary pretesticular, Receptaculum seminis usually absent. Vitellaria usually lateral. Postacetabular, sometimes extending in front of acetabulum. Uterus short or long intercaecal. Preovarian egg larg, 0.065-0.12 mm long. Excretory vesicle y-shaped with simple or chambered stem, bifurcating behind posterior testis into long cornua reaching anterior end with or without ramified branches. Parasitic in gut of fishes, reptiles, birds and mammals in bile ducts, ureter and urinary tubules of birds.

Type genus: *Echinostoma* Rudolphi, 1809

Family: Echinostomatidae, Poche, 1925

Genus: *Echinostoma* Rud, 1809

Species: *E. revolutum* Frolich, 1800

31 trematode parasites of this species were collected from the Bursa Fabricii of *Cattle egret*. These hosts were collected from Rampur Naikin, Sidhi in Nov. 2012.

Diagnosis: The body is elongated with both the ends pointed. It measures 4.6-89 mm. by 1 mm-1.9 mm. The narrowing of the region anterior to the ventral sucker being very slight until extremity of the larger collar is reached where the width is 1.0 mm diminishing more anteriorly being 0.8 mm. at the junction

of the collar.

The collar is large measures 2-0.8 mm. × 0.033-1.2 mm. the greatest breadth occurs in the mid region. The arrangement of spines is single row they are 34-27 in number. Body spines cover the body ventrally.

The oral sucker which measures 0.014 × 0.299 × 0.2 mm. is circular, sub terminal and relatively very small in comparison to ventral sucker. The front of the bowl shaped acetabulum is situated 0.98 mm. from the anterior end of the worm and measures 0.045-0.7mm. across by 0.06-0.07mm. in length. The short pre-pharynx is followed by a pharynx 0.2-0.5mm. wide long narrow oesophagus which is 0.2-0.3mm. divides directly in front of the acetabulum into two narrow simple intestinal crura. The testes median, close to one another, tandem entire, without lobulation or deeply lobulated. The anterior testis measures 0.37-0.5mm. × 0.3-0.5mm. and the posterior testis measures 0.3mm×0.5mm. the front of the anterior testis lying 5.3 mm. away from the anterior end of the parasite. The posterior edge of the posterior testis is 3.7 mm. to 3.92mm. away from the corresponding end of the worm. Cirrus pouch and associated structures occupy a very small area of 0.3mm. in diameter lying immediately behind the intestinal bifurcation and below the ventral sucker.

The ovary is pre testicular oval and measures 0.15-0.3mm.× 0.18-0.3mm. The oviduct is very small bends directly and almost immediately enters the ootype, which travels more or less at right angle to it behind the ovary. Shell gland is flattened out and occupies most of the region between anterior testis and the ovary. Laurer's canal is short. The uterine coils occupy the space between the testis and the acetabulum. Eggs measure 0.09×0.058 microns. The transverse yolk ducts cross nearly midway between the rear of the ovary and the front of the anterior testis to form a yolk reservoir near the middle of the shell gland and from this a common yolk duct travels obliquely forwards to enter the ootype near the anterior edge of the shell gland.

Host : *Cattle egret*

Location : Bursa fabricii

Locality : Rampur Naikin, Sidhi (M.P.)

Family : Echinostomatidae, Poche, 1925

Genus : *Pegasomum* Ratz, 1903

Species : *P. indicum* Saksena, 1960

58 trematode parasites of this species were collected from the Bile duct of *Bubulcus ibis*. These hosts were collected from Churahat, Sidhi in January. 2013.

Diagnosis: The body of the trematode is spindle shaped, measuring 3.7-4.1 mm. in length and 1.0-1.1 mm in breadth at the region of the acetabulum. The cephalic collar is poorly developed measuring 0.18 mm in length and 0.32-0.34 mm in breadth. The collar lappets do not meet ventrally. The whole body is covered with small backwardly directed spines while at the collar region the spines form a coronet. In the glycerine cleared specimen the body spines are clearly seen. The body spines at the level of the ovary measures 0.021 mm in length and 0.006 mm in breadth. While the biggest angular spine of the collar measures 1.015 mm in length and 0.003 mm in breadth. The ventral corner group of angular spines on each side consists of four bluntly pointed spines which are arranged in a single row. It is not possible to count the total number of

collar spines forming the coronet as they are poorly developed, leathery and small. They get mixed up with the musculature of the collar.

The oral sucker is poorly developed. It is triangular in shape measuring 0.08-0.10 mm in breadth. The ventral sucker is situated at a distance of 0.9-0.99 mm from the anterior end. It is almost spherical measuring 0.045-0.49 mm in diameter. The prepharynx is 0.1 mm in size. The oesophagus is 0.18-0.21 mm long. The intestinal caeca extend up to the posterior end of the body. The excretory pore lies at the posterior end of body. The leads into a Y-shaped excretory bladder. The genital pore is situated some distance below the intestinal bifurcation in the median line.

The gonads are located in the posterior half of the body. The anterior testis, situated at a distance of 1.99-2.3 mm from the anterior end, is boat shaped transversely placed covering nearly the whole breadth. It measures 0.76-0.88 × 0.049 mm size. The posterior testis is triangular in shape measuring 0.70-0.74 × 0.7-0.71 mm. The distance between the two testis is 0.01 mm. The cirrus sac measure 0.45-0.49 × 0.22-0.27 mm. It lies anterior to the acetabulum covering the area up to half the level of the acetabulum on its right side. It encloses vesicular seminalis, pars prostatica and the muscular cirrus.

The ovary is oval in shape lying above the anterior testis slightly towards the right side of the median line. It measures 0.21-0.25 × 0.17 – 0.18 mm. the shell glandmass lies on the left side of the ovary. The receptaculum seminalis is absent. The uterus arise from the anteriolateral margin of the shell glandmass and its coils are transversely placed in the intercaecal areas between the acetabulum and the ovary.

The vitelline glands consist of small follicles extending from the middle level of the pharynx to the posterior end of the body. From the pharynx to the anterior level of the acetabulum the vitelline unites in the median plane forming a continuous zone. Similarly below the posterior testis they are densely crowded and meet with each other. In the remaining portions the vitellaria lie in the lateral side of the body, covering the intestinal caeca completely. The transverse vitelline ducts are located in front of the anterior testis and meet the shell gland by a small common duct. The eggs are present in large numbers. They are oval in shape, measuring 0.072 × 0.057 mm in size.

Host : *Bubulcus ibis*

Location : Bile duct

Locality : Churahat, Sidhi (M.P.)

Family : Echinostomatidae Poche, 1925

Genus : *Paryphostomum* Dietz, 1909

Species : *P. dollfusi* Agrawal, 1958

29 trematode parasites of this species were collected from the Intestine of *Bubulcus ibis*. These hosts were collected from Kushmi, Sidhi in April 2012.

Diagnosis: Body short, median size measuring 11-08-13.36 in length and 1-95-2.56 in breadth. Aspinose, collar broad with 46 collar spines in a group of three on each side and 0.208-0.234 in breadth. Ventral sucker large, cup shaped measuring 1.2-1.38 in length and 1.0-1.19 in breadth. Ratio between suckers is 1:5. Pre-pharynx small. Pharynx muscular 0.247-0.52 long. Intestinal simple extending up to posterior end. The testes are deeply lobed, rosette shaped. Anterior testis

measures 0.60-1.15 in length and 0.49-0.68 in breadth, 5-7 lobed. Posterior testis measures 0.76-1.0 in length and 0.449-0.68 in breadth, 6-7 lobed, cirrus sac small dorsal to ventral sucker, obliquely disposed between intestinal bifurcation and ventral sucker. It measures 0.532-0.96 in length and 0.26-0.40 in breadth. Vesicular seminalis bipartite, occupying major portion of cirrus sac. Male genital aperture dorsal, median and just beneath the intestinal bifurcation.

Ovary non-lobulate pretesticular, situated in the middle of the body length and 0.68 in breadth. Receptaculum seminis present. Uterus with many coils and numerous egg. Egg measure 0.091-0.010 × 0.052, follicles extend from a little behind the ventral sucker to the posterior end of the body. The follicles lie laterally, and they approach towards the middle line in the post testicular region but do not become confluent.

Host : *Bubulcus ibis*

Location : Intestine

Locality : Kushmi, Sidhi (M.P.)

Family : Echinostomatidae Poche, 1925

Genus : *Paryphostomum* Dietz, 1909

Species : *P. bubulcusi* Agarwal, 1958

17 trematode parasites of this species were collected from the Intestine of *Bubulcus ibis*. These hosts collected from Bagdara, Sidhi in August 2013.

Diagnosis: Body medium sized 8.45-10.58 in length and 2.0-2.9 in breadth Aspinose. Collar kidney shaped with 47 collar spines arranged in a single row and uninterrupted by the oral sucker, end spines in group of three on each side and not larger than the marginal spines. Oral sucker larger than pharynx measuring 0.234-0.286 in length and 0.273-0.286 in breadth. Ventral sucker large cup shaped measuring 1.313-1.344 in length and 0.88-1.0 in breadth. Sucker ratio 1: 4.5. Prepharynx small. Pharynx thickly muscular, measuring 0.195-0.272 in length and 0.22-0.56 in breadth, oesophagus narrow, 0.39-0.576 in length. Caeca simple up to posterior border of ventral sucker, give out small sacculations there after and terminate blindly at the level of the arms of excretory bladder, testes much lobed and rosette shaped. Anterior testis measures 0.48-0.528 in length and 0.6-0.67 in breadth, 6 lobed further subdivided into 2-3 and each lobe smaller lobules. Posterior testis measures 0.482-0.52 in length and 0.52-0.64 in breadth, 6 lobed with each lobe further subdivided. Cirrus sac small, dorsal to ventral sucker, slightly obliquely disposed between intestinal bifurcation and the ventral sucker, measuring 0.2-0.416 in length in 0.312-0.32 in breadth. Vesicula seminalis bipartite, occupying major portion of cirrus sac, measuring 0.256-0.299 in length and 0.096-0.195 in breadth. Parsprostatica conspicuous. Male genital pore dorsal, median and just beneath the bifurcation of the intestinal caeca. No common genital sinus. Ovary faintly lobed situated in the middle of body length and measures 0.24-0.32 in length and 0.44-0.48 in breadth, uterus coiled with many eggs. Eggs measure 0.0321-0.052 in length and 0.091-0.096 in breadth. Female genital pore on ventral side a little beneath the bifurcation of intestinal caeca. Vitelline follicles commence from beneath the ventral sucker, extend laterally in the body, overlapping the intestinal caeca and the follicles of the two sides tend to approach the middle line of the body in the post testicular region but do not become

confluent.

Host : *Bubulcus ibis*
 Location : Intestine
 Locality : Bagdara, Sidhi (M.P.)
 Family : Echinostomatidae Poche, 1925
 Genus : *Echinoparyphium* Dietz, 1910
 Species : *E. indicum* Rai, 1961

42 trematode parasites of this species were collected from the Intestine of *Cattle egret*. These hosts collected from Bellan river, Sidhi in September 2013.

Diagnosis: Body slender, median sized, measuring 5.807-8.62 in length and 1.31-1.87 in breadth. Spines up to level of ovary. Collar kidney shaped having 38 collar spines arranged in two rows, uninterrupted. End groups of 4 spines on each side. Oral sucker measures 0.049-0.099×0.066-0.099. Ventral sucker pre-equatorial, measuring 0.322-0.392×0.274-0.392. Prepharynx present measuring 0.046-0.072 in length and 0.0003-0.009 in breadth. Pharynx well developed measuring 0.072-0.082 × 0.049-0.069. Oesophagus measures 0.066-0.171 in length and 0.003-0.009 in breadth. Intestinal caeca simple extending to a little above the posterior end of body. Testes tandem, smooth and ovoid. Anterior test is measuring 0.267-0.343×0.235-0.298. Posterior test is touching the posterior border of anterior test is or slightly away, measures 0.282-0.376×0.235-0.314. Cirrus sac oval, situated between intestinal bifurcation and acetabulum, measuring 0.148-0.33 in length and 0.082-0.138 in breadth. It includes large vesicula seminalis, small pars prostatica and long retractile cirrus. Cirrus non-spinous. Ovary median or slightly submedian, pretesticular, measures 0.094-0.133 × 0.109-0.141. Oviduct arises from the inner margin of the ovary. Receptaculum seminis post ovarian. Mehlis gland posterior to receptaculum seminalis. Eggs few in number (7-10) measuring 0.08-0.095 in length and 0.042-0.049 in breadth, vitelline follicles extending from the anterior level of anterior testis to a little above the posterior end of body. The follicles of two sides either distinctly separate or confluent at a few places in the post testicular region. The vitelline reservoir prominent, lying above anterior testis.

Host : *Cattle egret*
 Location : Intestine
 Locality : Bellan river, Sidhi (M.P.)
 Family : Echinostomatidae, Poche, 1925
 Genus : *Echinoparyphium* Ditz, 1910
 Species : *E. dollfusi*, Agarwal, 1959

71 trematode parasites of this species were collected from the Intestine of *Anhinga melanogaster*. These hosts were collected from Gopad river, Sidhi in March 2013.

Diagnosis: The body is small slender with lateral margins parallel measuring from about 2.09 to 3.15 mm. long and from about 0.36 to 0.53mm. in maximum breadth. Which is in the region of the ventral sucker. Surface of the body is without any spines.

The collar is distinctly kidney shaped and measuring from about 0.24 to 0.30 mm. in maximum breadth. Collar spines are thirty-four in number. They are arranged in two rows and are not interrupted dorsally. The spines of the oral row are distinctly than those of the aboral row and are arranged

alternately with them. On either side of the collar is a group of end spines each group having four large spines. The end spines are much larger than either the oral or the aboral spine. In one specimen the group of end spines has three large and one small odd spine on the side where as four uniformly large spine on the other side of the collar.

Oral sucker measures from 0.054 to 0.057mm. long and from 0.054 to 0.086mm. broad. Ventral sucker from 0.299 to 0.377mm. long and 0.260 to 0.364 mm. broad and is about five times the size of the oral sucker.

Mouth encircled by the oral sucker, prepharynx fairly long measuring 0.216 mm., pharynx conspicuous, measuring from 0.054 to 0.09mm. long and from 0.039 to 0.057 mm. broad. Oesophagus long and slender, intestinal caeca simple and extending to the posterior end of the body.

Testes situated behind the ovary, tandem and present an interesting series of stage with respect to their lobulation. Anterior testis measures from 0.234 to 0.338 mm. long and from 0.221 to 0.299mm. broad. It is mostly ovoid but indication of lobulation are also seen in a few specimens. Posterior testis measures from 0.285 to 0.337mm. long and from 0.221 to 0.299 mm. broad. Its outline varies from an ovoid to a distinctly four lobed condition and with a number of intermediate conditions. Cirrus sac small, anterior to the ventral sucker, measuring from 0.068 to 0.120mm. long and from 0.043 to 0.126 mm. broad. Vesicula seminalis undivided occupies major half of the cirrus sac and measuring 0.032 × 0.025 mm. pars prostatica absent. Cirrus proper is without any spines on its surface or at its base. Ovary situated in front of the testes, rounded and measuring from 0.078 to 0.104 by 0.065 to 0.130mm. Oviduct arise from the inner margin of the ovary, describes a loop at its origin and receives the duct from the transverse vitelline duct soon after forming the loop and finally enters into the ootype which is situated in the midline of the body. Receptaculum seminis is absent, from the ootype the uterus extends laterally to above the ovary and finally opens to the outside through the common genital pore which is situated a little below the point of intestinal bifurcation. The eggs are few in number (10 to 14) elliptical, large and measures from 0.078 to 0.091 by 0.052 to 0.065 mm.

Vitelline follicles take on a brownish tinge when treated with catechol solution. They commence from the level of the anterior margin of the anterior testis, extend laterally on the two sides of the body, completely overlapping the intestinal caeca and also passing in the intercaecal zone. In the post testicular region the vitelline follicles apparently converge towards the median line but remain separated presumably by the stem of the excretory vesicle. The vitelline follicles thus extend to the posterior tips of the intestinal caeca. They send out small ductules which unite with each other to form a transverse vitelline duct on each side of the body in front of the anterior testis. The two transverse vitelline ducts unite with each other in the median line and thus form a kind of a bridge just above the median line and anterior testis. Thus transverse ducts are uniformly thick and do not distinguish a vitelline reservoir at the point of their union with each other. A slender duct originates from the point of union of the transverse ducts and extends anteriorly to open into the oviduct.

Excretory bladder is of the usual *Echinostoma* type with the

excretory pore situated about 0.014 mm. above the posterior tip of the body.

4. Discussion & Conclusion

Numerous schemes have been suggested for the origin of the Digenea. Various of the world have derived the ancestral form from Monogenea, Aspidobothrea, and even insects. However, most authorities today believe that trematodes share an ancestor with some of the free-living flatworms, probably rhabdocoels. Whatever the ancestral digenean, any system of their phylogeny must rationalize the evolution of their complex life cycles in terms of natural selection, a most perplexing task.

Some more workers, in the forties, came in the field, notable among them being Baugh, S.C. (1958) ^[1] - Contribution to our knowledge of digenetic trematodes, Chauhan, B.S. (1963) ^[2] - Studies on the trematode fauna of India, Dwivedi, M.P. and Chauhan, B.S. (1970) ^[3] - On some Digenetic trematodes, La Rue, G.R. (1957) ^[4] The classification of Digenetic Trematoda. Mehra, H.R. (1957) ^[5] - The classification of digenetic trematode, Overstreet, R.M. (1969) ^[6] - Digenetic trematodes of marine teleost fishes from Biscayne Bay, Florida and Saksena, J.N. (1963) ^[7] - Studies on the morphology, cytology and early embryology of certain digenetic trematodes.

Digeneans display much more host specificity to their molluscan hosts than to their vertebrate hosts. This suggests that they may have established themselves as parasites of molluscs first and then added a vertebrate host as a later adaptation. It is not difficult to imagine a small, rhabdocoel-like worm invading the mantle cavity of a mollusc and feeding on its tissues. In fact, the main hosts of known endocommensal rhabdocoels are molluscs and echinoderms. The stage of the protodigenean in the mollusc was probably a developmental one, with a free-living, sexually reproducing adult. There are several reasons this is a reasonable hypothesis. First, a free reproductive stage would be of selective value in dispersion and transferral to new hosts. Precisely this lifestyle is shown by *Fecampia erythrocephala*, a rhabdocoel symbiont of various marine crustaceans. Second, the possession of a cercarial stage is surprisingly ubiquitous among digeneans, and most of these are adapted for swimming. Those without tails show evidence that the structure has been secondarily lost.

If one grants that the present adult represents the ancestral adult, which was free living, it is clear that additional (asexual) multiplication in the mollusc would have been advantageous also, and the alternation of the two reproductive generations could have been established. It is likely that such free-living adults would often be eaten by fish, and individuals in the population that could survive and maintain themselves in the fish's digestive tract for a period of time would have selective advantage in extending their reproductive life. *Fecampia erythrocephala*, for example, dies after depositing its eggs.

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